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Evaluation of bio-control agents and soil amendments against root rot incidence of mungbean incited by *Macrophomina phaseolina* (Tassi.) Goid

Nitisha Gahlot, RR Ahir, Abhinav and Sushila Choudhary

Abstract
Mungbean [*Vigna radiata* (L.) Wilczek] also referred to as 'golden gram' and is affected by number of diseases, of which the root rot is the one of serious diseases caused by *Macrophomina phaseolina.* Experiment was conducted in *kharif* 2017 at Department of Plant Pathology, SKN College of Agriculture, Jobner, Jaipur (Rajasthan). In this study four bio-control agents were evaluated for their antagonistic ability to suppress the growth of *Macrophomina phaseolina* both in the laboratory and pot conditions (Seed treatment, FYM enriched soil treatment and seed-cum-soil treatment of BCA). Five different soil amendments were used to examine their capacity to reduce the root rot disease incidence in pot conditions. Among the tested antagonists, *Trichoderma harzianum* showed maximum mycelial growth inhibition (83.55%) of the pathogen, followed by *Trichoderma viride* (78.11%) in *in vitro* condition. In pot conditions maximum disease control was recorded with seed-cum-soil application of *T. harzianum* (78.22 and 75.99%) followed by *T. viride* (72.22 and 70.21%) over control (54.80 and 63.85%) at 40 and 60 days after sowing, respectively. In soil amendments maximum disease control was recorded with neem cake (77.93 and 75.88%), followed by mustard cake (67.87 and 65.60%) over control at 40 and 60 days after sowing, respectively.

Keywords: Mungbean, root rot, *Macrophomina phaseolina*, bio-control agents, soil amendments

Introduction
Mungbean [*Vigna radiata* L. Wilczek] is one of India's most significant pulse crops. It is a legume crop that belongs to the *Fabaceae* family and the *Papilionaceae* subfamily. Moongbean also referred as celera bean, moong, green gram and golden gram. According to Vavilov (1926) [18] mungbean is a native of India and Central Asia. It is grown in these areas since prehistoric period. Mungbean is an excellent source of protein (24.5%) with high quality lysine (460 mg/g N), tryptophan (60mg/g N), carbohydrate (51%) and vitamins (3%). It has also significant amount of ascorbic acid (Vitamin-C) and when sprouted, it includes riboflavin (0.21mg/100g) and minerals (3.84g/100g) according to Gopalan *et al.* 1995 [8]. In India mungbean commonly grown in Rajasthan, Madhya Pradesh, Uttar Pradesh, Orissa, Maharastra, Karnataka and Bihar. It is grown about 23.36 lakh ha area with total production 13.90 lakh tones and productivity 438 kg/ha (Anonymous, 2016-2017) [2]. In Rajasthan, total area under mungbean is 22.4 lakh hectares with the production of 9.71 lakh tonnes and average productivity of 473kg/ha (Anonymous, 2017-18) [2]. It is mainly cultivated in arid and semi-arid districts including Nagaur, Jaipur, Jodhpur, Sikar, Pali, Jhunjhunu and Ajmer. Infection of *Macrophomina phaseolina* occurs primarily during the flowering and pod formation stage (Singh *et al*., 1990) [15] or during seed development stage (Trapero-Casas and Jimenez – Diaz, 1985) [17]. Yellowing of the leaves was the common indication of root rot disease and these leaves may drop off in two to three days. The plant may wilt within a week. At ground level, dark lesions on the stem can be noticed. When the plants are pulled out from the soil and examined, root rot symptoms can be seen on the basal stem and main roots. In advance stage on the affected tissues, scattered sclerotial bodies can be visible in the early stages (Singh and Srivastava, 1988) [14]. *Macrophomina phaseolina* survives in / on seed and persisted in the soil as black sclerotia which are generated in large number on infected host tissues and then disseminated in the soil during tillage activities (Sheikh and Ghaffar, 1978) [13]. Sharma and Singh (2001) [12] observed that *Rhizoctonia bataticola* infects 0.5-38% of mungbean seeds, resulting in losses of 10.85% grain yield and 12.3% protein content (Kaushik *et al*., 1987) [10]. Root rot incited by *Macrophomina phaseolina* (Tassi) Goid. Has been rated as most devastating disease of mungbean.

Management strategies of this disease include a large range of options but farmers largely depend on fungicides due to its higher control efficiency over other methods. However, wider use of fungicides can cause hazards to human health and known to increase environmental pollution. Therefore, to off sate this negative impact, alternative eco-friendly approaches for control of root rot of mungbean are needed.

Material and Methods

1. Evaluation of bio-control agents by dual culture method (*in vitro*)

Screening of two fungal (*Trichoderma harzianum, T. viride)* and two bacterial bio-control agents (*Pseudomonas fluorescens and Bacillus subtilis)* was done by dual culture technique (Dennis and Webstar, 1971) [3]. Twenty ml of autoclaved PDA was poured into in each sterilized Petriplate and allowed for solidification after 3 hours of pouring. These plates were inoculated with 5 mm diameter mycelial bit taken from 7 day old culture of *M. phaseolina* and antagonistic agents both were placed separately at equal distance on the periphery of Petriplates. PDA Petriplates inoculated with pathogen alone served as check. Inoculated Petriplates were incubated at 30+1 ºC in BOD incubator for 7 days. Linear growth of pathogen and bio-control agent was measured and per cent growth inhibition was recorded after 7 days of incubation. For efficacy of bacterial bio-control agent (*Pseudomonas fluorescens* and *Bacillus subtilis*) sterilized Petriplates containing 20 ml of NA (nutrient agar) were first inoculated with 7 days old culture of *M. phaseolina* and incubated at 30+1 ºC For 24 hrs. These plates were again inoculated with 5 mm disc of sterilized four filter paper dipped in suspension of bacterial bio-control agent. Four of these discs were placed at equal distance and incubated for 7 days at 30+1 ºC. The per cent growth inhibition was measured after 7 days incubation. Four replications of each treatment were maintained. Per cent inhibition of mycelia growth of the pathogen was calculated as per formula given by Vincent (1947) [19].

$$\text{Per cent growth inhibition} = \frac{C - T}{C} \times 100$$

Where

C = Diameter of the colony in check (average of both diagonals)

T = Diameter of colony in treatment (average of both diagonals)

2. Evaluation of bio-control agents in pot condition (*in vivo*)

Commercial formulation of four bio-control agents *Trichoderma harzianum*, *T. viride*, *Bacillus subtilis* and *Pseudomonas fluorescens* were tested in the pot by applying through seed, soil and seed-cum-soil inoculation methods. Four replications were maintained for each treatment. In all pot experiments, the inoculum, multiplied on sorghum grains was used @20 gm/pot at the time of sowing.

a. Bio-control agents applied through seeds

Before applying of bio-agents on the surface of seeds, the seeds of mungbean were moistened with 5 per cent gum solution (10 ml/kg seeds). Then, the seeds were treated with commercially available formulation of bio-agents @6g/kg seeds.

b. Bio-control agents applied through soil

Prior soil application, the commercially available formulations of bio-agents were multiplied on well rotten and moistened FYM for 10 days under shade then enriched FYM was incorporated into the pot at the time of sowing of mungbean.

c. Bio-control agents applied through seed and soil

The seeds of mungbean were treated with bio-agents (6 g/kg seeds) as mentioned in point (a) and then bio-agents also applied into soil with enriched FYM as mentioned in point (b). Fungus inoculated pots without treatment served as check. Observation on root rot incidence was recorded at 40 and 60 days after sowing. Per cent disease incidence was calculated as follows:

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants}} \text{ x100}$$

3. Evaluation of soil amendments applied through soil in pot condition

The experiment was carried out in 9x12 inches earthen pots. The soil amendments (Gypsum, poultry manure, vermicompost, mustard cake and neem cake) were thoroughly mixed as per recommended dose of nitrogen supplied by each manure in each pot @10 per cent (w/w) of soil before 1 month of sowing. The inoculum was added @20 g in each pot and mixed thoroughly up to 5-7 cm depth in the pot. In each pot 10 surface sterilized seeds were sown. Surface sterilized seed sown without organic manure with inoculated pot served a check. For each treatment four replications were maintained. Light watering was given at regular interval in each pot to maintain proper moisture levels. Percent disease incidence and percent disease control at 40 and 60 days after sowing, was calculated by using the formula

$$\text{Disease incidence (\%)} = \frac{\text{No. of diseased plants}}{\text{Total no. of plants}} \times 100$$

$$\text{Disease control(\%)} = \frac{\text{PDI in control} - \text{PDI in treatment}}{\text{PDI in inoculated control}} \times 100$$

Table 1: Treatment details

S. No.	Name of soil amendments	Quantity/ pot	Quantity/ha
1.	Neem cake	20G	8T/HA
2.	Gypsum	25G	10T/HA
3.	Musturd cake	20G	8T/HA
4.	Vermicompost	25G	10T/HA
5.	Poultry manure	25G	10T/HA

Result and Discussion

1. Evaluation of bio-control agents (*in vitro*)

Results (Table 2) indicated that all the bio-control agents *viz*., *Bacillus subtilis, T. harzianum, T. viride* and *Pseudomonas fluorescens* were antagonistic to the growth of *Macrophomina phaseolina*. Maximum mycelial growth inhibition (83.55%) of the pathogen was recorded with *T. harzianum* followed by *T. viride* (78.11%) and minimum mycelial growth inhibition was recorded with *Pseudomonas fluorescens* (44.20%) followed by *Bacillus subtilis* (40.25%).

Table 2: *In vitro*

Bio-agents	Per cent inhibition of mycelial growth*
Trichoderma harzianum	83.55 (66.07)
Trichoderma viride	78.11 (62.10)
Pseudomonas fluorescens	44.20 (41.67)
Bacillus subtilis	40.25 (39.38)
Control	00.00
S.Em+	0.94
CD (p=0.05)	2.91

*Average of four replications,
Figures given in parentheses are angular transformed values

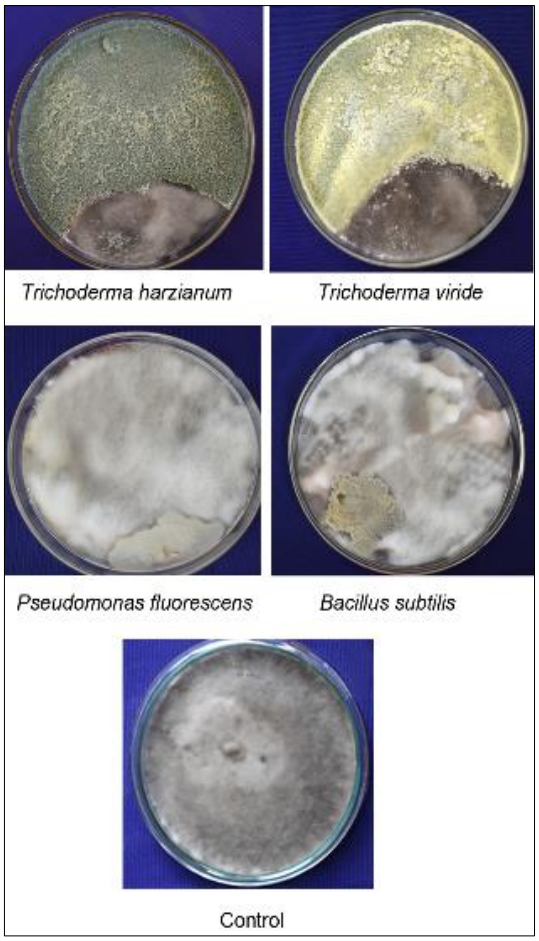

Fig 1: Evaluation of bio-agents against *Macrophomina phaseolina* by dual culture technique after 7 days of incubation at 30 + 1 ºC

2. Evaluation of bio-control agents in pot condition (*in vivo*)

a. Evaluation of bio-control agents applied through seed treatment (*In vivo*)

A perusal of data (Table 3) revealed that minimum disease incidence (24.03 and 26.82%) was recorded with seed application of *T. harzianum*, followed by *T. viride* (25.32 and 29.10%) as compared to control (53.75 and 65.15%) at 40 and 60 days after sowing respectively. Maximum disease control was observed with *T. harzianum* (55.29 and 52.69%) followed by *T. viride* (52.89 and 49.19%) over control at 40 and 60 days after sowing respectively.

Table 3: Evaluation of bio-control agents against root rot of mungbean applied through seed treatment (*in vivo*)

S. no.	Bio-agents	Dose	Percent disease incidence*		Percent disease control	
			40 DAS	60 DAS	40 DAS	60 DAS
1.	*Trichoderma harzianum*	6 g/kg	24.03 (29.35)	26.82 (31.19)	55.29	52.69
2.	*Trichoderma viride*	6 g/kg	25.32 (30.21)	29.10 (32.65)	52.89	49.19
3.	*Pseudomonas fluorescens*	6 g/kg	34.62 (36.04)	39.15 (38.73)	39.33	36.33
4.	*Bacillus subtilis*	6 g/kg	35.12 (36.34)	40.76 (39.68)	37.68	34.36
5.	Control	--	53.75 (47.15)	65.15 (53.82)	0.00	0.00
	S.Em+		0.85	1.03		
	CD (p=0.05)		2.62	3.16		

* Average of four replications
Figures given in parentheses are angular transformed values

b. Evaluation of bio-control applied through enriched FYM in soil (*in vivo*)

A perusal of data (Table 4) showed that minimum disease incidence (18.22 and 20.71%) was recorded with soil application of *T. harzianum*, followed by *T. viride* (24.46 and 26.57%) as compared to control (53.15 and 64.08%) at 40 and 60 days after sowing respectively. Maximum disease control was observed with *T. harzianum* (65.71 and 62.68%) followed by *T. viride* (53.97 and 51.31%) over control at 40 and 60 days after sowing respectively.

Table 4: Evaluation of bio-control agents against root rot of mungbean applied through enriched FYM in soil (*in vivo*)

S. no.	Bio-agents	Dose	Percent disease incidence*		Percent disease control	
			40 DAS	60 DAS	40 DAS	60 DAS
1.	*Trichoderma harzianum*	6kg/ha	18.22 (25.27)	20.71 (27.07)	65.71	62.68
2.	*Trichoderma viride*	6kg/ha	24.46 (29.64)	26.57 (31.03)	53.97	51.31
3.	*Pseudomonas fluorescens*	6kg/ha	30.31 (33.40)	33.52 (35.38)	42.97	41.44
4.	*Bacillus subtilis*	6kg/ha	32.42 (34.71)	38.30 (38.23)	39.00	37.10
5.	Control	--	53.15 (46.81)	64.08 (53.18)	0.00	0.00
	S.Em+		0.83	1.00		
	CD (p=0.05)		2.57	3.10		

* Average of four replications
Figures given in parentheses are angular transformed values

c. Evaluation of bio-control agents applied through seed-cum- soil treatment (*in vivo*)

A perusal of data (Table 5) revealed that minimum disease incidence (11.66 and 13.33%) was recorded with seed cum soil application of *T. harzianum*, followed by *T. viride* (15.22 and 16.02%) as compared to control (54.80 and 63.85%) at 40 and 60 days after sowing. Maximum disease control was observed with *T. harzianum* (78.22 and 75.99%), followed by *T. viride* (72.22 and 70.21%) over control at 40 and 60 days after sowing respectively.

Table 5: Evaluation of bio-control agents against root rot of mungbean applied through seed-cum-soil method (*in vivo*)

S. no.	Bio-agents	Dose (ST+SA)	Percent disease incidence*		Percent disease control	
			40 DAS	60 DAS	40 DAS	60 DAS
1.	*Trichoderma harzianum*	6 g/kg + 6 kg/ha	11.66 (19.97)	13.33 (21.41)	78.22	75.99
2.	*Trichoderma viride*	6 g/kg + 6 kg/ha	15.22 (22.96)	16.02 (23.59)	72.22	70.21
3.	*Pseudomonas fluorescens*	6 g/kg + 6 kg/ha	26.75 (31.14)	29.78 (33.07)	51.18	48.66
4.	*Bacillus subtilis*	6 g/kg + 6 kg/ha	27.25 (31.47)	30.54 (33.55)	50.27	47.47
5.	Control	-	54.80 (47.75)	63.85 (53.04)	0.00	0.00
	SEm+		0.89	1.04		
	CD (p=0.05)		2.74	3.20		

* Average of four replications
Figures given in parentheses are angular transformed values
Where, ST= Seed treatment, SA= Soil application

3. Evaluation of soil amendments applied through soil in pot condition (*in vivo*)

It is evident from the data (Table 6) that all the evaluated soil amendments significantly reduced root rot incidence of mungbean over check. Neem cake was most effective over all other treatments resulted minimum disease incidence (12.30 and 15.23%) as compared to check (55.25 and 63.15%) followed by mustard cake (17.25 and 21.72%). Maximum disease control over check was recorded with neem cake (77.93 and 75.88%), followed by mustard cake (67.87 and 65.60%) over control at 40 and 60 days after sowing, respectively. Our observations are in agreement with the findings of Hussain *et al*., (1990) [9] who found *Trichoderma harzianum* effective in controlling the infection of *R. solani* in mungbean. Deshmukh and Raut (1992) [5] reported that in pot trials *T. harzianum* and *T. viride* were effective in inhibiting the mycelial growth of *R. solani* in mungbean and in reducing the disease incidence. Nagamma *et al.* (2012) [11] found *Trichoderma harzianum* most effective in controlling of disease followed by *Bacillus subtilis* and *T. viride* as compared to control. Deshmukh *et al.* (2016) [4] evaluated potentiality of bio agents *Trichoderma harzianum* and *Pseudomonas fluorescens* for management of rot rot of mungbean caused by *Macrophomina phaseolina* in which maximum inhibition was achieved by *Trichoderma harzianum*.

In soil amendments application to the soil, our findings are in line with the results of Tiyagi and Alam (1995) [16] found that oil cake of neem reduced frequency of pathogenic fungi *Macrophomina phaseolina*, *Rhizoctonia solani* including *Phyllosticta phaseolina* and *Fusarium oxysporum* f. sp. *ciceri* significantly. Dubey (2002) [7] in his experiment of soil application with five per cent Neem cake showed best performance as it increased seed germination and grain yield of mungbean and decreased seedling mortality and disease intensity of web blight. Dhingani *et al.* (2013) [6] tested four organic extracts like neem cake, castor cake, mustard cake and FYM in which maximum mycelium inhibition was showed by neem cake (59.40%) followed by FYM (42.56%) against root rot of chickpea in *in vitro*.

Table 6: Evaluation of soil amendments against root rot of mungbean applied through soil (*in vivo*)

S. no.	Organic amendments	Dose	Percent disease incidence*		Percent disease control*	
			40 DAS	60 DAS	40 DAS	60 DAS
1.	Vermicompost	25 gm	27.36 (31.54)	32.56 (34.79)	50.47	48.44
2.	Gypsum	25 gm	26.30 (30.85)	30.34 (34.04)	52.39	51.95
3.	Neem cake	25 gm	12.30 (20.53)	15.23 (23.76)	77.93	75.88
4.	Poultry manure	25 gm	33.62 (35.44)	40.20 (39.35)	39.14	36.35
5.	Mustard cake	20 gm	17.75 (24.92)	21.72 (28.47)	67.87	65.60
6.	Control	--	55.25 (48.01)	63.15 (52.62)	0.00	0.00
	S.Em+		0.83	0.94		
	CD (p=0.05)		2.54	2.91		

* Average of four replications,
Figures given in parentheses are angular transformed values

Conclusion

Biological control is of much significance in view of hazards caused by toxic chemicals or in a situation where pathogens develop resistance to fungitoxicants. Incorporation of soil amendments in to the soil improves structure and texture of the soil. These exert positive impact on plant growth by changing aeration, porosity temperature and water holding capacity of the soil which results in rapid root extension, balance availability of nutrients and better plant vigour. All these changes indirectly reduce the incidence of root rot disease.

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